^{13}C CPMAS NMR spectroscopy of organic matter transformation in ligno-cellulosic waste products composted and vermicomposted *(Eisenia fetida andrei)*

Marthe Vinceslas-Akpa and Michelle Loquet

Laboratoire d'Écologie, Université de Rouen, 10, bd. de Broglie, 76821 Mt.St.Aignan Cedex, France.

Abstract

Lignocellulosic wastes (of maple) were composted for ten months under controlled conditions. Chemical analyses and ^{13}C CPMAS NMR spectroscopy were carried out, in order to study the transformation of the organic matter.

At first, the bulk of organic matter and polysaccharides, including cellulose, underwent a relatively rapid decomposition. The degradation of aromatic compounds and lignin occurred only after one month of composting. The rapidity of this process was at its greatest during the following three months.

A polycondensation and/or a neosynthesis mechanism is observed in the last stages. The two types of compost evolved differently, a higher level of aromatic compounds and polysaccharides occurring in the vermicompost, yet characterized by a lower aromaticity rate . The C/N ratio decreases, reflecting the changes in the carbon fractions as well as a higher level of nitrogen in the vermicompost.

Keywords: Earthworm, *Eisenia fetida*, composting, lignocellulose, organic matter, ^{13}C CPMAS NMR spectroscopy.

Résumé

Des déchets lignocellulosiques d'érable, après mise en compostage et lombricompostage d'une durée de 10 mois, sont analysés par dosage chimique et par spectrométrie ^{13}C CPMAS RMN du solide en vue d'étudier l'évolution de la matière organique.

Dans une première phase, la matière organique totale et les polysaccharides totaux, dont la cellulose, subissent une dégradation relativement rapide. Les aromatiques totaux et la lignine ne se dégradent qu'après un mois de compostage. Le taux de dégradation le plus important pour ces composés est observé pendant les trois mois suivants.

L'évolution du compost et celle du lombricompost se différencient dans la phase finale, essentiellement par la mise en place d'un mécanisme de polycondensation et/ou de néosynthèse plus actif dans le lombricompost. Il se traduit par un enrichissement plus net en produits aromatiques et en polysaccharides et néanmoins un taux d'aromaticité inférieur. Le rapport C/N diminue, reflétant l'évolution des fractions carbonées ainsi qu'un enrichissement azoté supérieur dans le lombricompost.

Mots-clés : vers de terre, *Eisenia fetida*, compostage, lignocellulose, matière organique, spectrométrie ^{13}C CPMAS RMN.

INTRODUCTION

The storage of pruning wastes results in a product, the disposal of which poses a serious problem for local authorities. Composting, the biological oxydation of organic matter, is an useful process which could be improved by the combined action of earthworms and microflora living in the intestine and the environment.

Eisenia fetida andrei (Bouche) is often utilized for vermicomposting (Bouche, 1972; Satchell, 1983; Albanell, 1988; Hervas, 1989).

In nature, worms help to accelerate the transformation of organic matter, by aeration and bioturbation, by their excreta and qualitative or quantitative influence upon telluric microflora (Bachelier, 1963; Loquet, 1972; Loquet *et al.*, 1977; Rouelle *et al.*, 1985).

Moreover the intestinal mucus which consists of easily metabolizable compounds is considered to be a mediator of a priming-effect between earthworms and soil microflora (Martin *et al.,* 1987; Barois, 1987). Couture *et al.* (1983) showed that soil macrofauna play an important role as a regulating agent in the immobilization of mineral nitrogen and in ligninolytic activity. Neuhauser and Hartenstein (1978 a and b) working on the reactivity of soil macro-invertebrates peroxydases with lignins and lignin compounds suggested: firstly, that those enzymes may be used in part to enhance the polymerization of aromatic compounds and thus perhaps increase humification; secondly, that none of the investigated invertebrates (including *Eisenia fetida*) was able to degrade native lignin, even with the gut microorganisms present, though they were instrumental in demethylating and cleaving various aromatic compounds as vanillic and cinnamic acids.

The aim of the present study was to analyse the transformation of organic matter in ligno-cellulosic maple waste by composting and by vermicomposting in controlled conditions using standard dosages and solid state ^{13}C-CPMAS NMR spectroscopy. This method , which permits the identification of different molecular types, is a non destructive technic and should be considered as a powerful tool for the investigation about the decomposition of organic matter. It has been recently utilized to study the molecular structure of humic substances (Hempfling *et al.,* 1987; Wilson, 1987; Schnitzer, 1991; Cameron *et al.,* 1992), the changes occuring during the composting of grape marc and quantify different carbons (Inbar *et al.,* 1991).

MATERIAL AND METHODS

Experimental procedure

In this study, each container hold 100 g of fresh pruning waste products air dried; the thickness of the layer was almost 8 cm as *Eisenia fetida* utilized for vermicomposting is an epigeic worm.

The woody material was previously crushed in order to homogenize it and make the substrates accessible to microbial attack. In vermicomposting experiments ten clitellate adult Eisenia fetida were added for 100 g of pruning wastes.

The samples were incubated for one year, in an incubation chamber in which temperature was maintained at 18°C and moisture content at 80 % in equilibrium with the atmosphere of the aerated containers. In both series three containers were sacrificed each month and an average sample of 100 g sent to the laboratory for analysis, (refered as: compost = C, vermicompost = LC).

Chemical study

All samples were previously powdered to ensure homogeneity and were not sieved to keep the whole organic matter. Different quantitative analyses were carried out in triplicate, each month:
- weight loss;
- organic matter determined by loss on ignition at 550°C;
- organic carbon (Anne method);
- total nitrogen by Kjeldahl analyzer;
- insoluble cellulose by a modified Sharrer method (in Monties, 1980);
- lignin following the method of Johnson (1961) by spectrometry at 280 nm and respecting special anhydrous conditions (Van Zyl, 1978);
- microcalorimetry of wastes with reference to benzoic acid calorific power (6,319 cal/mg);
- CPMAS ^{13}C NMR solid state spectra were obtained on an AM 400 Brucker spectrometer at the Chemistry Department of the University of Rouen.

Operating conditions were:
SF frequency = 100,6 MHZ
wr spinning rate = 4230 HZ
P2 contact time = 1 ms
Aq acquisition time = 35 ms
D1 relaxation time = 4 s.

In order to eliminate spinning side bands, spectra were recorded with Toss sequence. They were cut off at 190 ppm because no peak of interest appeared between 220 and 190 ppm. The experimental conditions were nearly those related by Inbar *et al.* (1991) studying the organic matter transformation of solid wastes from wineries.

The different samples were analyzed under the same experimental conditions to allow a comparative interpretation. The spectra obtained were normalized in order to compare successive stages of decomposition.

The spectra were divided into the following regions (Inbar *et al.,* 1991): 0-50 ppm (alkyl C), 50-100 ppm (O-alkyl C in carbohydrates and aliphatic structures bearing OH groups), 100-112 ppm (non-protonated C in tannin, anomeric C), 112-145 ppm (aromatic C), 145-165 ppm (phenolic C), 165-190 ppm (C in carboxyl groups) and 190-215 ppm (carbonyl C). the subdivisions of the spectra follow closely the commonly used scheme (Beyer *et al.,* 1993).

Particular peaks were measured:
55 ppm, methoxy of aromatic C (Almendros *et al.,* 1992; Ramunni *et al.,* 1992).
63 ppm, C6 of cellulose (Haw, 1984).
89 ppm, C4 of cellulose (Haw, 1984);
74-75 ppm, C2-C3-C5 of cellulose (Haw, 1984);
105 ppm, C1 of polysaccharides (Haw, 1984).

The proportions of different types of carbon were determined by measuring peak areas.

Table 1. – Chemical composition of lignocellulosic wastes.

Organic Matter by ignition %	96,2
Total carbon %	44,7
Nitrogen °/$_{oo}$	7,2
Insoluble cellulose %	43
Lignin %	18,7
Total Saccharides %	12
C/N	62

Total aromaticity was calculated by expressing aromatic C (110- 165 ppm) as percentage of aliphatic C (0-110 ppm) and aromatic C:

$$\frac{\text{aromatic C}}{\text{aromatic C} + \text{aliphatic C}} \times 100$$

(Hatcher in Inbar *et al.*, 1991).

CHEMICAL EVOLUTION OF WASTES

The initial composition of lignocellulosic wastes is reported in *Table* 1. The major contituants are cellulose (43 %) and lignin (18.7 %) and the C/N ratio is 62.

Chemical analysis. Results

Total weight loss (table 2)

Total weight loss is about 35 % for vermicompost (LC) and 42,8 % for compost (C). However the first month of composting leads to a greater decrease in sample LC than in C: the result of a better primary mineralization of substrates. In both cases the monthly loss of weight is negligible after seven months.

Table 2. – Weight evolution of lignocellulosic wastes during composting.

Months	Compost	Vermicompost
0	100 g	100 g
1	82,5	78,4
2	73	76,2
3	66,8	70
4	64	66,8
5	61	66,7
6	60	65
7	59	64,3
8	58	65,7
9	57,2	65
Loss/9 month	42,8 %	35 %
Loss/1st month	17,5	21,6

Organic matter s.l.

The evolution is quantified in terms of weight loss of organic matter (*fig.* 1), of total carbon (*fig.* 2) and evolution of C/N ratio (*fig.* 3).

– The loss of organic matter, for the first month, was:

26 % in sample LC

20,7 % in sample C
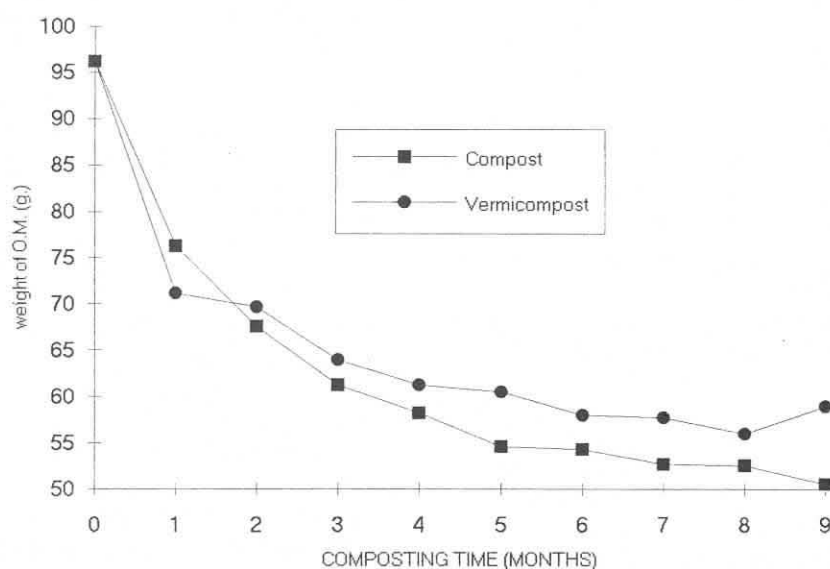
corroborating the observation above.

Then biodegradation of organic matter stabilizes in both composts but at a higher level in the vermicompost with even an increase after 8 months.

– The first month of composting shows a decrease of total carbon weight (*table* 3):

24,4 % in sample LC

18,6 % in sample C,

a phenomenon also observed for total organic matter.
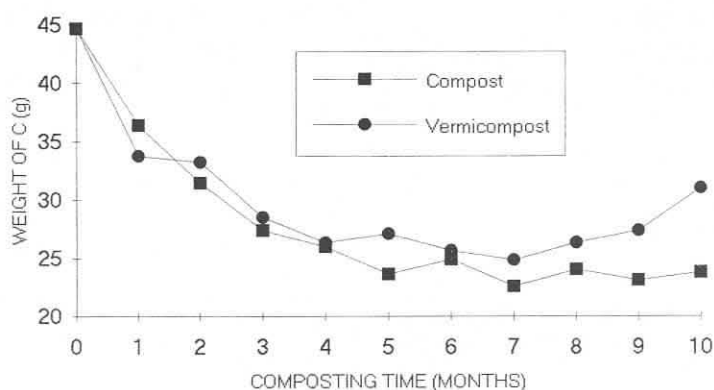

Figure 1. – Evolution of organic matter.

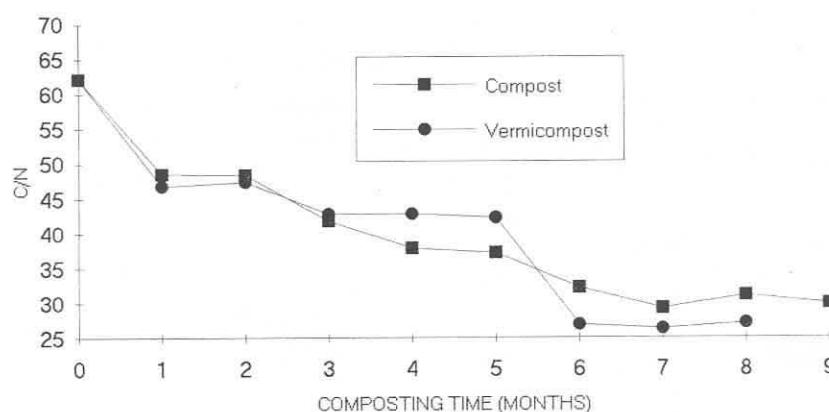**Figure 2.** – Evolution of carbon.

Figure 3. – Evolution of C/N ratio.

Table 3. – Evolution of carbon (%-weight-% of loss).

Composting time	Compost			Vermicompost		
	c %	weight g	% of loss	c %	weight g	% of loss
initial sample	44,7	44,7		44,7	44,7	
1st month	44,1	36,38	18,6	43,05	33,75	24,4
2nd month	43,1	31,46	29,6	43	33,22	25,7
7th month	38,3	22,6	49,4	38,7	24,88	44,3
9th month	40,4			42,1		

This loss is observable during seven months and thereafter stabilizes in the compost C, whereas in the vermicompost, the weight and percentage of carbon increase after this period.

– The C/N ratio, which is one of the most widely used indices for compost maturation, decreases in the compost from 62 to 30, while in the vermicompost it decreases from 62 to 27 (*fig.* 3). This change can be explained partly by an increase in total nitrogen in both samples after five months of incubation (*fig.* 4, *table* 4) and partly by carbon decrease, particularly in sample C.

However it is interesting to note that the increase in nitrogen is far more pronounced in samples with earthworms, probably due to nitrogen excretion and

stimulated activity of N-fixing bacteria (Bhatnagar, 1975; Rouelle, 1977; Couture and Fortin, 1983; Daniel and Anderson, 1992). Concerning nitrogen excretion, it was not possible to determine exactly which part origined from mucus secretion and urine. This impossibility has been note by Heine and Larink (1993).

Table 4. – Evolution of nitrogen in the compost and vermicompost.

Composting time	Compost			Vermicompost		
	N °/oo	weight g	% of Variation	N °/oo	weight g	% of Variation
Initial sample	7,2	0,72		7,2	0,72	
5th month		0,63	– 12,5		0,64	– 11
9th month	13,5	0,77	22	14,8	0,97	51,5

Cell wall polymers : cellulose (fig. 5) *and lignin* (fig. 6)

As those two polymers are the major constituants of woody wastes, their evolution was studied apart.

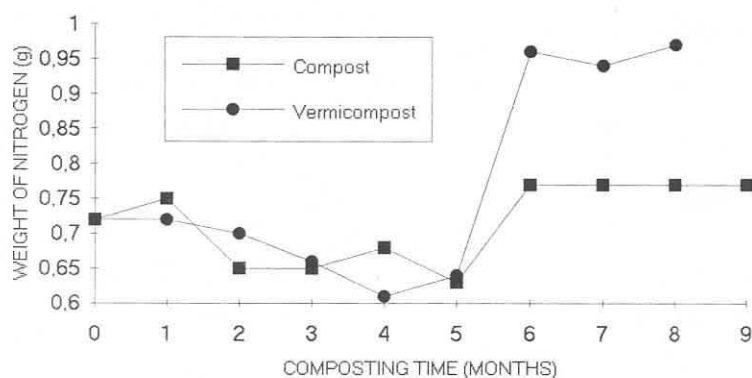Comparison of curves shows a slightly better cellulolysis in the compost, whereas, as expected, the
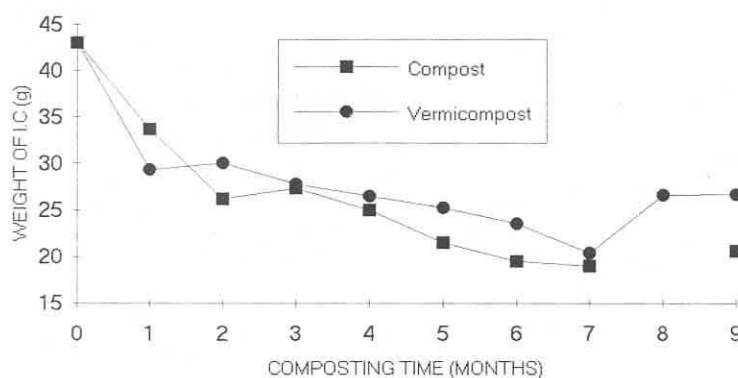
Figure 4. – Evolution of nitrogen.

Figure 5. – Evolution of insoluble cellulose.

value of cellulose loss is superior after the first month in the vermicompost (*table* 5, *fig.* 5). Unexpectedly, the organic fraction identified as being cellulose increases particulary at the end of vermicomposting (weight and percentage). The same results were found by Inbar *et al.* (1991) during composting of solid wastes from wineries.

The same changes occur for lignins. They are characterized by an expected increase of percentages, due to the efficient decomposition of other cell wall

polymers, and an unexpected increase of absolute amount after eight months, as shown in *Table* 6 and *Figure* 6. This one being more important in samples LC.

CPMAS ^{13}C NMR results

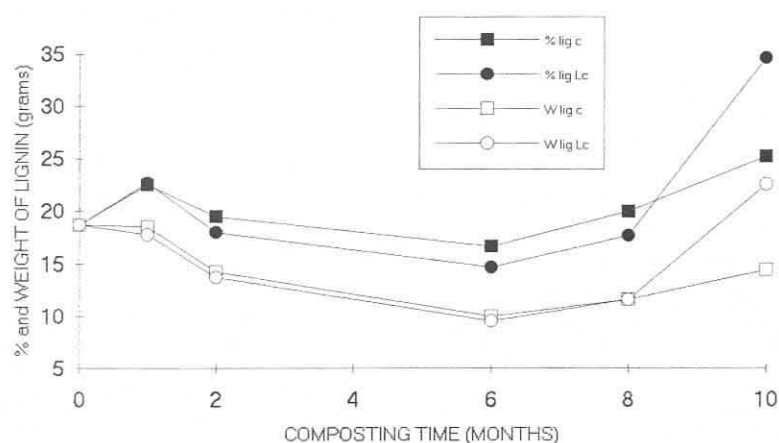Chemical analyses were complemented by NMR solid state spectroscopy.

Figure 6. – Evolution of lignin.

Table 5. – Evolution of insoluble cellulose.

Composting time	Compost			Vermicompost		
	(1)	(2)	% of loss	(1)	(2)	% of loss
Initial sample	43	43		43	43	
1st month	40,8	33,6	21,8	37,4	29,3	31,8
7th month	32,2	19	55,8	31,7	20,4	52,8
8th month	–	–		40,4	26,6	–
9th month	36,1	20,6		41	26,6	–

(1): % of insoluble cellulose; (2): weight g.

Table 6. – Evolution of lignin.

Composting time	Compost			Vermicompost		
	(1)	(2)	% of loss	(1)	(2)	% of loss
Initial sample	18,7	18,7		18,7	18,7	
2nd month	19,5	14,2	– 24	18	13,7	– 28
6th month	16,7	10	– 46,5	14,7	9,5	– 49,2
7th month	14,7	8,7	– 53,5	22,7	14,6	–
9th month	25,2	14,4	–	34,7	22,5	–

(1): % of lignin; (2): weight g.

NMR spectra (fig. 7)

This figure shows three spectra, selected from the different ones obtained. They represent respectively the control, the ten-month-old compost (C) and vermicompost (LC).

Evolution of organic matter

The aliphatic fractions, globally considered, decrease during composting (*fig.* 8 B) in both series (C and LC). This decrease is greater in vermicompost during the first month, but later on, the highest

significant loss is observed in compost (C), as noted for the "insoluble cellulose" fraction. In order to improve this analysis, the intensity of particular signals has been measured in spectra: 74-75 ppm, 105 ppm, 89 ppm, 63 ppm. All these peaks decrease during composting, the peak at 89 ppm disappearing at 5 months of vermicomposting (*fig.* 7).

Figure 11 represents peaks at 74-75 ppm and 105 ppm, and shows that cellulose and polysaccharide degradation is greater in the first six months of vermicomposting. The degradation continues in compost, whereas peak intensities increase in
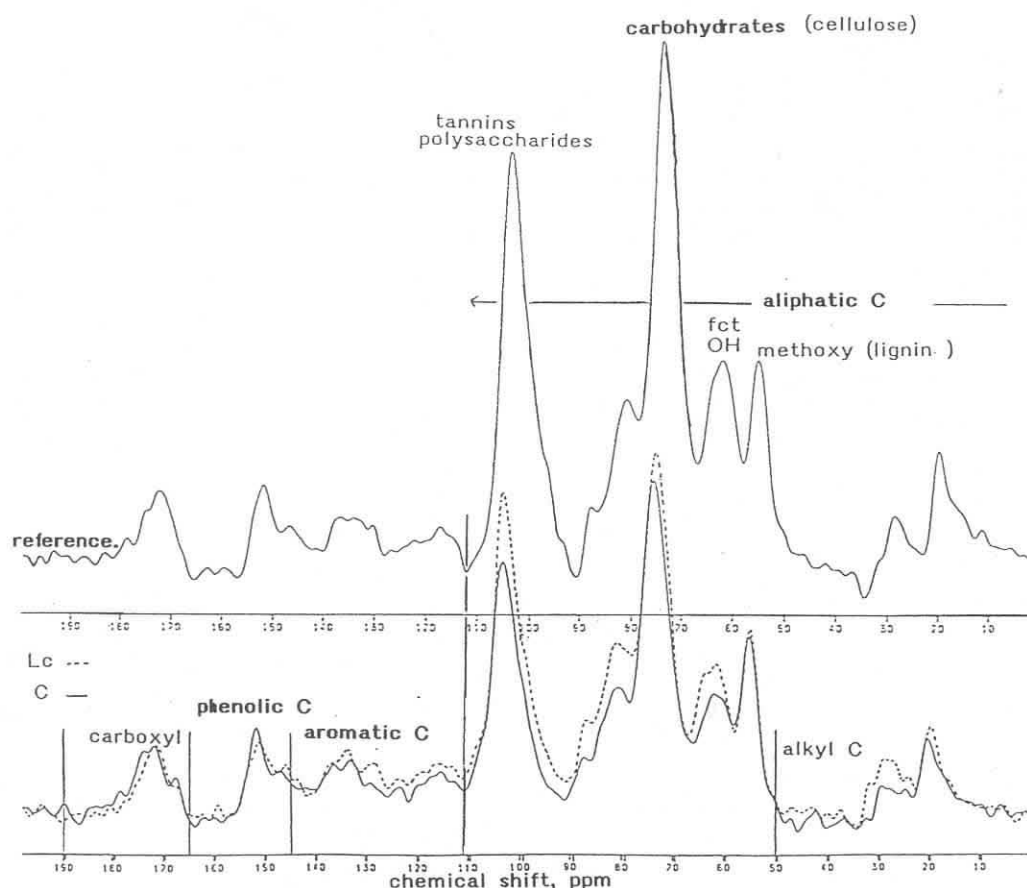
Figure 7. – NMR spectra reference and 10th month of composting.

vermicompost: the curves representing total aliphatic C evolution confirm this phenomenon (*fig.* 8 B).

Total aromaticity was calculated to give an overall view of the evolution of aromatic and aliphatic carbon. *Table* 7 and *figure* 8 show the values at 1, 5, 6, 8 and 10 months of composting.

Aromaticity increases in both series during the incubation time, the value increases by 33 % in LC and by 44 % in C (*fig.* 8 E). Firstly, this increase reveals essentially the degradation of non aromatic cell-wall compounds.

The curves of aromaticity obtained experimentally were compared with the theoretical curve. Theoretical values were calculated supposing that no degradation of lignin occured during composting time; it means that in the ratio:

$$\frac{\text{aromatic C}}{\text{aromatic C} + \text{aliphatic C}}$$

aromatic C remained at the initial value while aliphatic C decreased. The comparison of the three curves shows an effective degradation of the aromatic compounds (*fig.* 9). It occurs in both composts but it is more
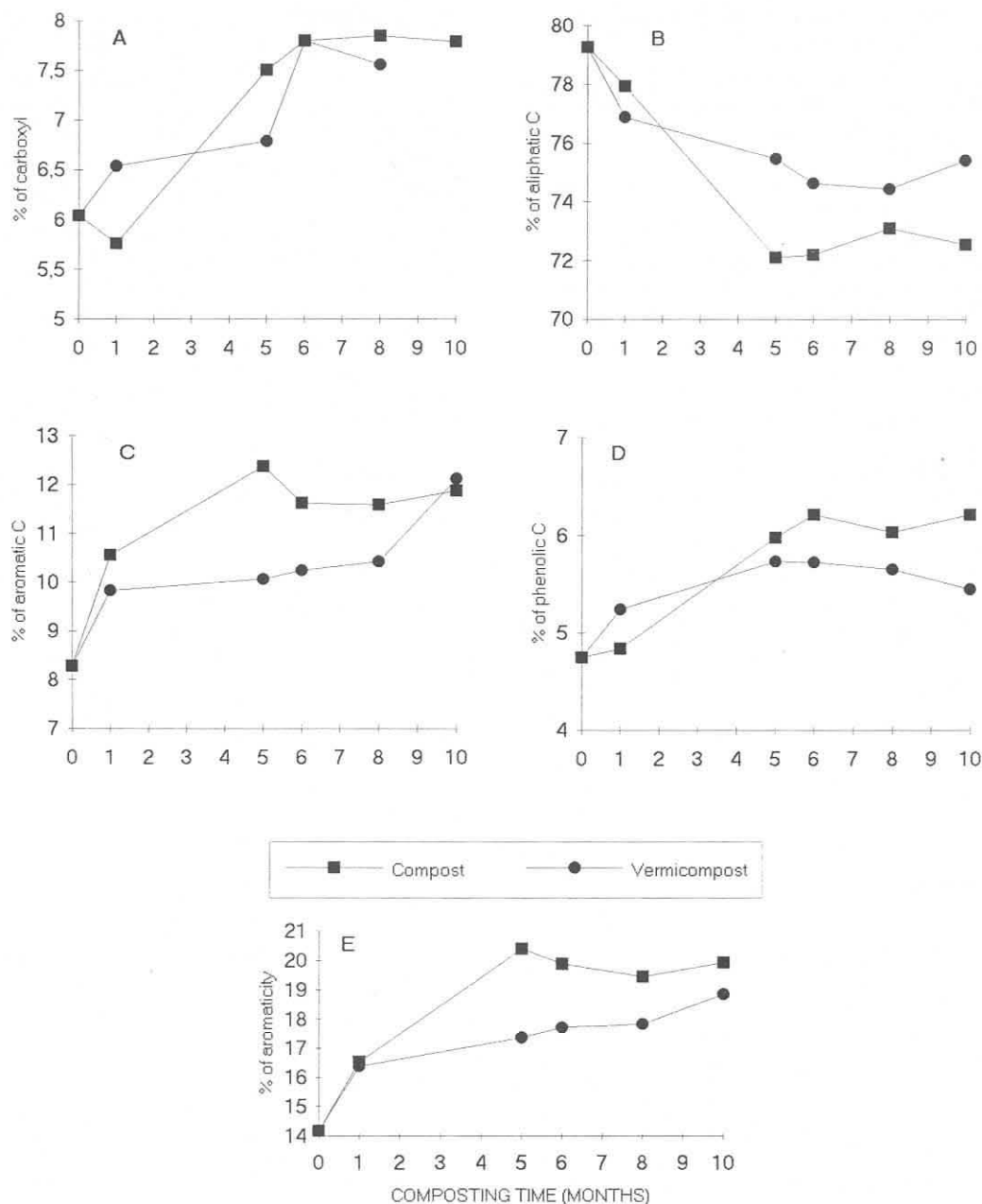

Figure 8. – Evolution of different fractions (NMR).

Table 7. – Evolution of total aromaticity during composting.

% of	Initial sample	1st month		5th month		6th month		8th month		10th month	
		C	LC	C	LC	C	LC	C	LC	C	LC
aliphatic C	79,26	77,94	76,87	72,11	75,45	72,19	74,62	73,10	74,43	72,54	75,40
total aromatic C	13,08	15,44	15,06	18,48	15,87	17,92	16,06	17,67	16,16	18,00	17,51
aromatic C S.S.	8,28	10,56	9,83	12,37	10,07	11,63	10,25	11,59	10,43	11,88	12,12
phenolic C	4,75	4,84	5,24	5,98	5,73	6,21	5,72	6,03	5,65	6,21	5,45
carboxyl	6,04	5,76	6,54	7,51	6,79	7,80	7,80	7,85	7,56	7,79	5,90
aromaticity	14,17	16,54	16,39	20,40	17,38	19,89	17,71	19,46	17,84	19,94	18,85

efficient in vermicomposting with *Eisenia fetida*, as shown by the lower percentages.

Moreover it is interesting to confirm by this method the enrichment in aromatic C on and after the 8 months, especially in the vermicompost.

The methoxy signal at 55 ppm represents lignin or by-products' demethylation (Almendros, 1992). It is one of the first stages of lignin decomposition (Oades *et al.*, 1987; in Baldock *et al.* 1992). It greatly decrease in both series (C and LC) after eight months (*fig.* 10). It could be compared with the evolution of aromatic C studied by NMR analysis (*fig.* 8 C) and with the lignin values obtained either by chemical analysis (*fig.* 6) or by estimation from the NMR spectra following a procedure suggested by Haw *et al.* (1984) (*fig.* 10).

During the first seven months the lignin loss weight is effective in both composts as shown in *figure* 6. According to *figure* 10 the compost C is characterized by more important lignin percentages. As the degradation of polysaccharides, including cellulose is better in the compost LC (*fig.* 11) one can assume a more efficient ligninolysis in this compost. Parallely it is interesting to notice an increase of aromatic C values after 8 months of experiment mainly in the vermicompost (*fig.* 8 C), as observed for the lignin fraction quantified by Johnson's method.

DISCUSSION

As told before, the main constituants of pruning waste products are cellulose and lignin. The evolution of those wastes depends greatly on the degradation of those polymers, particularly lignins which are heteropolymeric molecules composed mainly of singly or doubly methoxylated phenyl-propyl phenolic units, linked through a variety of non-hydrolysable bonds. The heterogeneity of this compound and its very complex 3-dimensional structure explain its resistance to microbial attack. Moreover, the incrustation of lignin in a lignin-carbohydrate complexe can affect its biodegradability by non polysaccharides degrading microorganisms (Monties, 1980; Odier *et al.,* 1981). Consequently in this paper, lignin degradation concerns "in situ" lignin, so called by Monties (1980), and not prepared lignin. Yet, the biodegradation of this polymer is a fundamental stage in the carbon cycle and in the mechanism of humification. A great deal of research concerns above all fungal strains activity, however bacterial ligninolysis is also an important process (Kawakami *et al.* in Crawford, 1981; Odier and Monties, 1977-1978; Loquet and Vinceslas, 1987). The introduction of *Eisenia fetida* has permitted the comparison between composting and vermicomposting
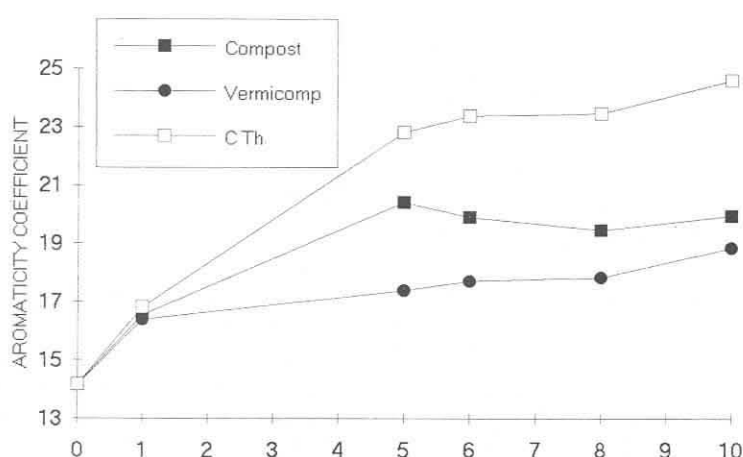
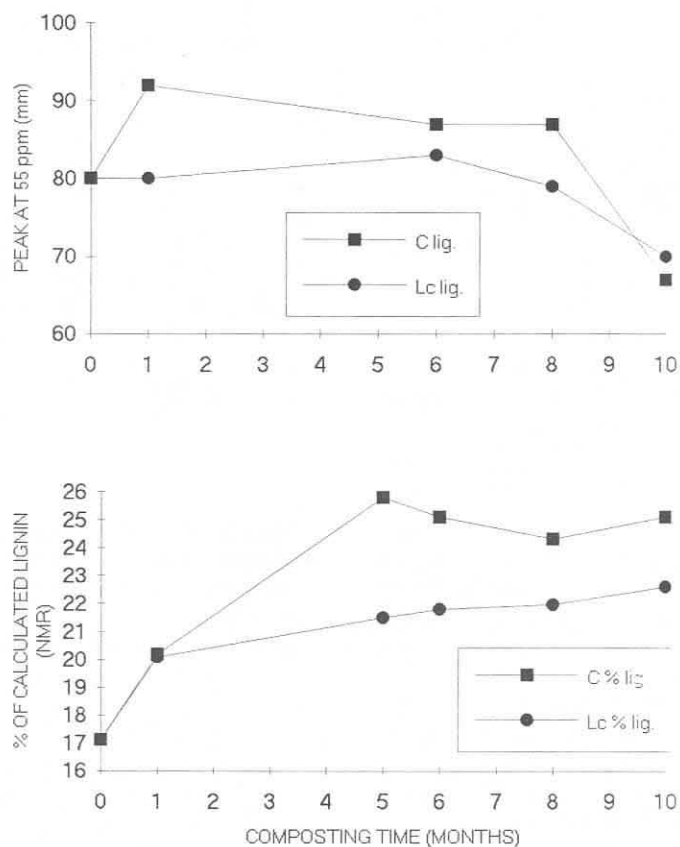**Figure 9.** – Evolution of aromaticity coefficient.

Figure 10. – Lignin evolution (NMR). C: compost; Lc: vermicompost.
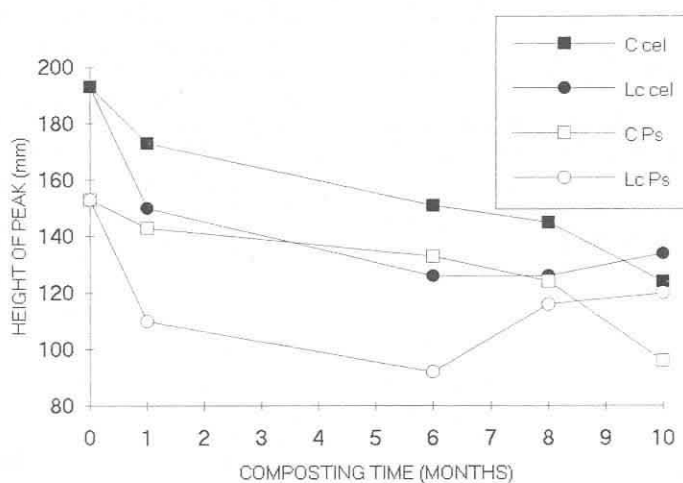

Figure 11. – Evolution of polysaccharides (NMR). Ps: polysaccharides; cel: cellulose; Lc: vermicompost; C: compost.

which may reflecte the synergy: microorganisms-pedofauna in the humification process.

Generally speaking, organic fraction degradation is greater during the first month of vermicomposting as shown by weight loss (17.5 % in C, 21.6 % in LC), organic matter loss (20.7 % in C, 26 % in LC), total carbon loss (18.6 % in C, 24.4 % in LC). This

phenomenon is observed again in some fractions: total aliphatic C, cellulose and lignin evolution.

The change in the C/N ratio accounting for the humification mechanism decreases from 62 to 27 in LC and to 30 in C. The difference between compost and vermicompost relates more to an increase in nitrogen content than to changes in carbon content. Indeed chemical analyses of total carbon, lignin and
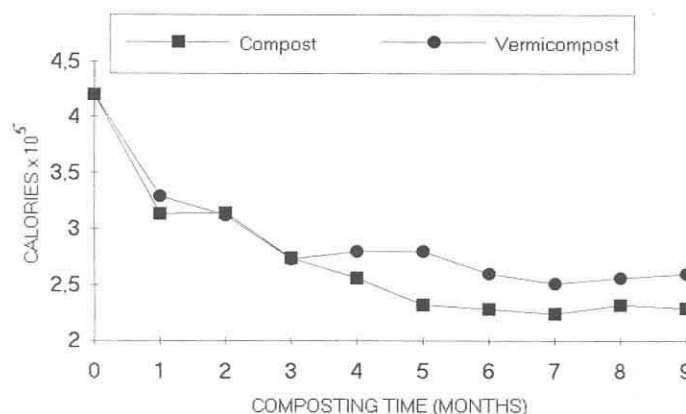
Figure 12. – Evolution of energy quantity in composts.

"insoluble cellulose" show an increase in the last stages of the vermicomposting experiment. These results are to be compared to those of Martin (1991) studying the influence of the endogeic tropical earthworm *Millsonia anomala* on soil organic matter. He observed antagonistic effects which vary with time: in the short term (a few hours), this species accelerates the mineralization while in the longer term (a few months), it reduces the decomposition of the soil organic matter by a protection due to microbial polysaccharides and other organic products during gut transit.

Late increases of "insoluble cellulose" and lignins can be partly understood thanks to NMR analysis, which results apparently invalidate the chemical data. Effectively according to NMR results:

– polysaccharides including cellulose undergo a more rapid and greater degradation in vermicompost;

– a net ligninolysis occurs throughout the composting time as confirmed by methoxy peak evolution and NMR-lignin values;

– a net production of new aromatic compounds takes place at the end of the composting period as well as a neosynthesis of polysaccharides particularly in the vermicompost.

The polycondensation postulated is in agreement with a study by Bailly and Raboanary (1986) showing that soil microorganisms can produce para-humic substances, if grown on simple phenolic acids. Trojanoski *et al.* (1987), investigating solubilization and polymerization of extracted lignins by several wood-inhabiting fungi, indicate that fungi secreting phenoloxidases, not only produce water soluble degradation products of lignin but can also polymerize low-molecular weight lignin.

Moreover Beyer *et al.* (1993) suggest that earthworms and collembola digest long chains of polysaccharides enhancing microbial colonization, simultaneously structure of lignins change, probably due to microbial oxidation and demethylation. They report that, according to Stott and Martin, the aromatic rings of lignin are cleaved by microbial enzyme

and should be present in soil organic matter as polysaccharides and humins. Barois *et al.* (1993) studying transformation of soil structure showed that there were processes of biodegradation and humification during gut transit.

The better mineralization of organic waste material in vermicompost, followed by a higher polycondensation are in agreement too with results of Rafidison (1982). This author found an oxidation of beech leaf polycondensates in the gut content of the anecic worm *Nicodrilus velox* as well as an increase in polycondensation in the casts.

The better ligninolysis, (see lignin percentages calculated with Haw's method), the increase of total aromatic C and polysaccharides, at the end of the experiment particularly in the vermicompost corroborate the results of the mentioned authors.

The results concerning lignin biodegradation, particularly in vermicompost, may explain the de-colorization of ligno-cellulosic wastes when incubated with gut homogenates and the associated microflora (Loquet and Vinceslas, 1987). This decolorization, typical of white-rot fungi metabolism, is interpreted as an efficient ligninolysis associated with phenol oxidase activity (Freitag *et al.*, 1992).

The energy condensed in composts was studied by microcalorimetry. The decrease of energy during the first three months reveals substrate mineralization, then the two curves differ and express the balance between biodegradation and synthesis activity as shown by *figure* 12. The energy reserve is greater in vermicompost, in agreement with the hypothesis of a more efficient restoration of the organic pool, moreover characterized by a lower percentage of aromaticity. The fact that organic matter evolves differently in compost and vermicompost can be explained partly by the mutualistic relationship between soil microflora, ingested microorganisms and intestinal mucus (Martin *et al.*, 1987; Trigo and Lavelle, 1993).

On the basis of the data presented in this study, a concomitance of a net enhancement of a biodegradation with significant neosynthesis (as shown for example by the increase of polysaccharides fraction at the end of vermicomposting time) and/or insolubilization resulting in a more important synthesis of organic compounds (humification), is confirmed in the vermicompost as well as a better ligninolytic activity.

REFERENCES

Albanell E., Plaixats J. and Cabrero T. (1988). – Chemical changes during vermicomposting *(Eisenia fetida)* of sheep manure mixed with cotton industrial wastes. *Biol. Fertil. Soils,* **6,** 266-269.

Almendros G., Martinez A. T., Gonzalez A. E., Gonzalez-Vila F. J., Fründ R. and Lüdemann H. D. (1992). – CPMAS 13C NMR study of lignin preparations from wheat straw transformed by five lignocellulose-degrading fungi. *J. Agric. Food Chem.,* **40 (7),** 1297-1302.

Bachelier G. (1963). – *La vie animale dans les sols.* Ed. ORSTOM, Paris. 279 pp.

Bailly J. R. and Raboanary M. (1986). – Sur la formation de substances parahumiques à partir d'acides phénoliques simples par des microorganismes du sol. *Agrochimica,* **vol XXX (1-2),** 58-74.

Baldock J. A., Oades J. M., Waters A. G., Peng X., Wassallo A. M. and Wilson M. A. (1992). – Aspects of the chemical structure of soil organic materials as revealed by solid-state 13C NMR spectroscopy. *Biogeochemistry,* **16,** 1-42.

Barois I. (1987). – Interactions entre les vers de terre *(Oligochaeta)* tropicaux géophages et la microflore pour l'exploitation de la matière organique du sol. *Unpublished doctorale thesis.*

Barois I., Villemin G., Lavelle P. and Toutain F. (1993). – Transformation of the soil structure through Pontoscolex corethrurus *(Oligochaeta)* intestinal tract. *Geoderma,* **56,** 57-66.

Beyer L., Schulten H. R., Fruend R. and Irmler U., (1993). – Formation and properties of organic matter in a forest soil, as revealed by its biological activity, wet chemical analysis, CPMAS ^{13}C-NMR spectroscopy and pyrolysis-field ionization mass spectrometry. *Soil Biol. Biochem.,* **25 (5),** 587-596.

Bhatnagar T. (1975). – Lombriciens et humification: un aspect nouveau de l'incorporation microbienne d'azote induite par les vers de terre. *Biodegradation et humification,* Kilbertus G., Pierron, Sarreguemines.

Bouche M. B., (1972). – *Lombriciens de France. Écologie et systématique.* INRA, Paris: 671 pp.

Cameron D. F. and Sohn M. L. (1992). – Functional group content of soil and sedimentary humic acids determined by CP/MAS ^{13}C NMR related to conditional Zn^{2+} and Cd^{2+} formation constants. *The Science of the Total Environment,* **113,** 121-132.

Couture M. and Fortin J. A. (1983). – Rôle de la pédofaune au sein du processus de dégradation de la matière ligneuse dans deux humus forestiers. *Rev. Écol. Biol. Sol.,* **20 (2),** 207-220.

Crawford R. L. (1981). – Lignin biodegradation and transformation, John Wiley and Sons, 154 pp.

Daniel O. and Anderson J. M. (1992). – Microbial biomass and activity in contrasting soil materials after passage through the gut of the earthworm *Lumbricus rubellus* Hoffmeister. *Soil Biol. Biochem.* **24 (5),** 465-470.

Deschamps A. M., Gillie J. P. and Lebeault J. M. (1981). – Direct delignification of bark chips with mixed cultures of bacteria. *European J. Appl. Microbiol. Biotechnol.,* **13,** 222-225.

Freitag M. and Morrell J. J (1992). – Decolorization of the polymeric dye Poly R-478 by wood-inhabiting fungi. *Can. J. Microbiol.,* **38,** 811-822.

Haw J. F. and Maciel G. E. (1984). – Carbon-13 nuclear magnetic resonance spectrometric study of wood and wood pulping with cross polarization and magic-angle spinning. *Anal. Chem.,* **56,** 1323-1329.

Heine O. and Larink O. (1993). – Food and cast analyses as a parameter of turn-over of materials by earthworms *(Lumbricus terrestris L.). Pedobiologia,* **37,** 245-256.

Hempfling R., Ziegler F., Zech W. and Schulten H. R. (1987). – Litter decomposition and humification in acidic forest soils studied by chemical degradation, IR and NMR spectroscopy and pyrolysis field ionization mass spectrometry. *Z. Pflanzenernaehr. Bodenk.,* **150,** 179-186.

Hervas L., Mazuelos C., Senesi N. and Saiz-Jimenez C. (1989). – Chemical and physico-chemical characterization of vermicomposts and their humic acid fractions. *The Science of the Total Environment,* **81/82,** 543-550.

Inbar Y., Chen Y. and Hadar Y. (1991). – Carbon-13 CPMAS NMR and FTIR spectoscopic analysis of organic matter transformations during composting of solid wastes from wineries. *Soil Science,* **152 (4),** 272-282.

Inbar Y., Hadar Y. and Chen Y. (1992). – Characterization of humic substances formed during the composting of

solid wastes from wineries. *The Science of the Total Environment,* **113,** 35-48.

Johnson D. B., Moore W. E. and Zank L. C. (1961). – The spectrophotometric determination of lignin in small wood samples. *Tappi,* **44 (11),** 793-798.

Kakezawa M., Nishida T. and Takahara Y. (1992). – Structural characteristics of humic acids extracted from woody composts by two-step composting process. *Soil Sci. Plant. Nutr.,* **38 (1),** 85-92.

Kawakami H., Sugiura M. and Kanda T. (1975). – Biodegradation of components of pulp waste effluents by bacteria. I. On the degradation of kraft lignin. *Tappi,* **29,** 309-315 .

Loquet M. (1972). – Étude de l'activité microbiologique d'une prairie permanente: le Haras du Pin. Comparaison avec les turricules de vers. *Rev. d'Écol. Biol. du Sol,* **11,** 47-65.

Loquet M., Bhatnagar T., Bouche M. B. and Rouelle J. (1977). – Essai d'estimation de l'influence écologique des lombriciens sur les microorganismes. *Pedobiologia.* **17,** 400-417.

Loquet M. and Vinceslas M. (1987). – Cellulolyse et ligninolyse liées au tube digestif d'*Eisenia fetida andrei* Bouché. *Rev. Écol. Biol. Sol,* **24 (4),** 559-571.

Martin A., Cortez J., Barois I. and Lavelle P. (1987). – Les mucus intestinaux de ver de terre moteur de leurs interactions avec la microflore. *Rev. Écol. Biol. Sol,* **24 (4),** 549-558.

Martin A. (1991). – Short- and long-term effects of the endogeic earthworm Millsonia anomala (*Omodeo*) (*Megascolecidae, Oligochaeta*) of tropical savannas, on soil organic matter. *Biol. Fertil. Soils,* **11,** 234-235.

Monties B. (1980). – Les polymères végétaux. Gauthier-Villars, Paris, 345 pp.

Neuhauser E. F. and Hartenstein R. (1978). – Reactivity of soil macroinvertebrates peroxidases with lignins and lignin model compounds. *Soil Biol. Biochem.,* **10,** 341-342.

Neuhauser E. F., Hartenstein R. and Connors W. J. (1978). – Soil invertebrates and the degradation of vanillin, cinnamic acid, and lignins. *Soil Biol. Biochem.,* **10,** 431-435.

Odier E. and Monties B. (1977). – Activité ligninolytique *in vitro* de bactéries isolées de paille de blé en décomposition. *C.R. Acad. Sci.* Paris, **284,** 2175-2178.

Odier E. and Monties B. (1978). – Biodégradation de la lignine de blé par *Xanthomonas. Ann. Microbiol.,* **129,** 361-377.

Odier E., Janin G. and Monties B. (1981). – Poplar lignin decomposition by Gram-negative aerobic bacteria. *Applied and Environmental Microbiology,* **41,** 337-341.

Rafidison G. Z. (1982). – Rôle de la faune dans l'humification: transformations des feuilles de hêtre par un ver anécique (Nicodrilus velox). *Unpublished doctorale thesis,* 104 pp.

Ramunni A., Pignalosa V. and Amalfitano C. (1992). – The lignin input in the structure of the humic acids from a farm-yard manured soil as detected by FT-IR, UV-visible and 13C NMR CP-MAS spectroscopy. *Agrochimica,* **36 (3),** 268-281.

Rouelle J. (1977). – Écologie du cycle de l'azote, dénombrements des peuplements microbiens des structures du sol (turricules et galeries) résultant de l'activité des vers de terre d'une prairie permanente de la plaine dijonaise. *Unpublished doctorale thesis,* 51 pp.

Rouelle J., Pussard M., Randrimamonjizaka J. L., Loquet M. and Vinceslas M. (1985). – Interactions microbiennes (bactéries et protozoaires), alimentation des vers de terre et minéralisation de la matière organique. *Bull. Ecol.,* **16 (1),** 83-88.

Satchell J. E. (1983). – Earthworm ecology from Darwin to vermiculture. *Chapman and Hall,* London.

Schnitzer M. (1991). – Soil organic matter, the next 75 years. *Soil Science,* **151 (1),** 41-58.

Trigo D. and Lavelle P. (1993). – Changes in respiration rate and some physicochemical properties of soil during gut transit through *Allolobophora molleri* (*Lumbricidae, Oligochaeta*). *Biol. Fertil. Soils,* **15,** 185-188.

Trojanowski A., Milstein O., Majcherczyk A., Haars A. and Hüttermann A. (1987). – Solubilization and polymerization of lignin by several wood-inhabiting fungi. In: *Lignin enzymic and microbial degradation,* Colloque INRA Paris, 23-24 avril 1987, **40,** 302 pp.

Van Zyl J. D. (1978). – Notes on the spectrophotometric determination of lignin in wood samples. *Wood Sci. Technol.,* **12,** 251-259.

Wilson M. A. (1987). – *NMR techniques and applications in geochemistry and soil chemistry.* Pergamon Press Oxford, England, 340 pp.